

Determination of fat content in foods by analytical SFE

FRED J. ELLER* and JERRY W. KING

Food Quality and Safety Research, National Center for Agricultural Utilization Research, Agricultural Research Service/US Department of Agriculture, ‡ 1815 North University Street, Peoria, IL 61604 USA

Analytical supercritical fluid extraction (SFE) is finding widespread application in the analysis of foodstuffs, agriculturally derived materials, and natural products. The high efficacy that SFE demonstrates toward the removal of oils and fats from such matrices makes it a natural technique for the determination of their fat and oil content. In this review, we have highlighted what is essential experimentally to optimize the SFE of fat and other lipid moieties from meats, oilseeds, snack foods, and cereal products. The role of extraction pressure/temperature and sample preparation before SFE is particularly emphasized as well as the inclusion of sorbents into the extraction process for controlling the molecular specificity of the extraction or the coextraction of water. Application of analytical SFE for total and speciated fat determination is cited for both home-built as well as both manual and automated commercial instrumentation. Finally, an appraisal of the future of the technique is made with respect to its acceptance as an official analytical method and the crucial interface between the end-user and the instrumentation companies that produce the analysis modules.

Keywords: SFE; fat; lipid; analysis; food

Introduction

Fat: definition and determination

Interest in dietary fat is widespread. Consumers, industrial food processors, and governmental agencies all have an intense interest in fat, although for very different reasons. Consumers are concerned with the reduction in the intake of total fat, saturated fat, and cholesterol for improving human health (Chao *et al.*, 1991). Fat control is necessary for food processors who must strive to meet consumer demands for products containing less fat (i.e. "fat-free" or "low-fat" foods) as well as to maintain costs and to comply with labeling requirements. Governmental agencies must have suitable methods for fat determination to assure accurate labeling of food products. Although the determination of fat content is one of the most common analyses performed in a foodstuffs laboratory, the quantitative extraction and analysis of fat is far from straightforward (Lumley and Colwell, 1991). With the ever-increasing range of processed, composite, and novel foods available, the analyst faces an increasingly difficult task of selecting an appropriate method for fat determination.

Impact of NLEA

As a result of the Nutritional Labeling Act (NLEA) of 1990, total fat is currently defined as the sum of all fatty acids obtained from total lipid extract expressed as triglycerides (Federal

*To whom correspondence should be addressed.

‡Names are necessary to report factually on available data, however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

Register, 1993a). The NLEA protocol consists of the following steps: 1) a hydrolytic treatment; 2) solvent extraction of lipids; and 3) the preparation of fatty acid methyl esters (FAMES) for gas-liquid chromatography (GLC) and the quantitation of saturated and unsaturated fat after stoichiometric conversion of FAMES to triglycerides. To date, however, there is only one method (i.e. House *et al.*, 1994), approved by the Association of Official Analytical Chemists International (AOAC), using this definition of fat.

According to the NLEA definition, fat determination begins with the isolation of the lipid. Although the terms fat and lipid are often used interchangeably, they are not equivalent. The classical definition of lipid refers to any of various substances that are soluble in organic solvents including mono-, di-, and triglycerides, free fatty acids, phospholipids, sterols, lipoproteins, waxes, and hydrocarbons (Maxwell, 1987; Lumley and Colwell, 1991). This definition of lipids could include organic soluble substances such as carbohydrates (i.e. sugars or starches) and amino acids or peptides (Lumley and Colwell, 1991). Clearly a lipid extract could contain much more than NLEA would define as fat.

There are two types of lipids, free and "bound," which together constitute total lipid. Free lipid components often occur in storage tissue and are easily extracted, if the sample is dry and well ground to enable solvent penetration (Lumley and Colwell, 1991). Free lipid may be determined by a simple extraction with a nonpolar solvent (e.g. hexane), whereas total lipid may involve a combination of solvents (e.g. nonpolar and polar) (Finney *et al.*, 1976) or sample hydrolysis/digestion (McGhee *et al.*, 1974) to extract bound lipid. Although fat derived from meat potentially contains some polar lipids (Maxwell, 1987), bound lipids are generally associated with cereal grains (Inkpen and Quackenbush, 1969; McGhee *et al.*, 1974). Whether free or bound lipids are extracted, the extract will almost certainly contain components that do not fit the NLEA definition of fat (Lumley and Colwell, 1991).

Uncertainties in "fat" determination

Historically, and even currently, many methods determine fat (more properly lipid) content by gravimetric measurements. Although these methods have been in use a long time, their accuracy is questionable, and they do not provide any information on the types of fats present, such as saturated fats (which is required by the NLEA for food labeling). There are a number of gravimetric methods available for measuring fat, however, the material that is extracted and called fat is dependent on the particular method used (Carpenter *et al.*, 1993). Current methods furnish extracts that are dependent on the solvent used, the extraction time, the type of food, the particle size, the temperature of extraction, and the proportion of lipid classes present (McGhee *et al.*, 1974; Maxwell, 1987). Because the extraction of lipids depends on the solvent used, the choice of solvent is one of the most critical steps in the determination of fat (Finney *et al.*, 1976; Hubbard *et al.*, 1977). Solvents such as petroleum ether, diethyl ether, and tetrachloroethylene extract triglycerides, but not phospholipids or free fatty acids, potentially underestimating fat content (Carpenter *et al.*, 1993). Although hexane and petroleum ether do not extract starches, protein, or water, diethyl ether is a better solvent for fat (Lumley and Colwell, 1991). However, ether extraction methods may underestimate the amount of fat in some bakery products (Hertwig, 1923), and diethyl ether has the disadvantage that it will absorb water.

On the other hand, it has been recognized for some time that some extracts do not contain pure triglycerides (Lepper and Waterman, 1925). More polar solvents such as chloroform-methanol extract triglycerides as well as mono- and diglycerides, phospholipids, terpenes, waxes, and nonlipid materials (McGhee *et al.*, 1974; Hubbard *et al.*, 1977). Inkpen and Quackenbush (1969) reported that the polar solvent (chloroform-ethanol-water) extracted substantial amounts of

nonlipid material from wheat products and that this solvent did not extract bound lipids as shown by subsequent acid hydrolysis of the residue. Hagan *et al.* (1967) reported that a solvent extract of an acid-hydrolyzed meat sample contained nonfat material in addition to the fat. Acid hydrolysis before ether extraction may lead to high fat (gravimetric) values due to the extraction of sugar or sugar byproducts (McGhee *et al.*, 1974), especially for grain products. Inkpen and Quackenbush (1969) recognized that even though a given solvent may yield an equivalent amount of total lipid as that obtained using a hydrolytic treatment, it does not constitute evidence of complete extraction of total lipids by such solvents.

Hydrolysis methods

The direct extraction of composite foods (e.g. egg noodle or bread) with ether gives fat values considerably less than those for the combined fat of the ingredients composing the product (Hertwig, 1923). Apparently the ether does not penetrate the glutenous particles sufficiently to extract all the fat (Hertwig, 1923). Disintegration of the sample with an acid and heat hydrolyzes the proteins and starch, disrupts the plant cell walls, and liberates the fat, so as to allow its easy extraction (Hertwig, 1923). There are many methods using hydrolysis before actual extraction. The purpose of the hydrolysis/digestion is to release bound lipids (Inkpen and Quackenbush, 1969). Acids, bases, and enzymes are used to hydrolyze proteins, polysaccharides, and complex lipids to make the fats more available to the solvent. The acid method of Hertwig (1923) hydrolyzed phospholipids and recovered practically all of the fat contained in the component materials (Hertwig, 1923). A description of common hydrolysis methods for the analysis of fat from food have been summarized from Lumley and Colwell (1991) and Carpenter *et al.* (1993). A tabulation of the more prominent methods are as follows.

Weibull-Berntrop

This procedure involves hydrochloric acid digestion, passing the digest through filter paper, drying the filter paper, extracting the filter paper in a soxhlet-type extractor, evaporating the solvent, and obtaining a subsequent gravimetric determination. Although this method is useful for releasing bound lipids from composite products, it has many manipulative steps, it uses large amounts of solvents, and acid digestion may produce nonfat ether extractables.

Werner-Schmid

Like the Weibull-Berntrop method, this procedure involves hydrochloric acid digestion. However, the fat is extracted directly from the digest using Mojonnier-type flasks. This method also uses large amounts of solvents, and acid digestion may produce nonfat ether extractables.

Roese-Gottlieb and Mojonnier

The Roese-Gottlieb and Mojonnier methods are often used for the extraction of fat from dairy products and involve the precipitation and solubilization of protein by ethanol and ammonia, respectively, and solubilization fats in petroleum ether and diethyl ether. The Roese-Gottlieb method cannot be used for some cheese because the protein is not soluble in ethanolic ammonia and some fatty acid moieties are not extracted from the ammoniacal phase by the petroleum ether-diethyl ether mixture. This method also uses large amounts of solvents, acid digestion may produce nonfat ether extractables, and not all fat is extracted from the ammoniacal phase.

Folch and Bligh-Dyer

These procedures involve the homogenization of the sample mixture with a mixture of chloroform:methanol:water. Several extraction, washing, and filtration steps are involved. The ratio of chloroform:methanol:water is critical for quantitative lipid extraction and to avoid the extraction of nonlipid materials.

Fat level in the matrix and the impact of minor lipid constituents

The contribution of nonfat materials can become a serious issue in low-fat products (Carpenter *et al.*, 1993). An extraction after hydrolysis of low-fat bakery goods may yield a relatively high amount of sugars but very little actual fat.

On the other hand, for products known to contain only small amounts of polar lipids (e.g. phospholipids) or bound lipids (i.e. total lipids essentially equal to free lipids) it may be reasonable to ignore their contributions and skip extraction with a polar solvents or a hydrolysis step. For example, the phosphatide content of rapeseed is only 0.1%. However, the phosphatide content of beef brain is 6.1% (Swern, 1964) and constitutes a significant portion of the total lipid extract.

Gravimetric methods that give statistically "equivalent" results may actually have extracted different amounts of fat, as well as different amounts of "other" materials (e.g. sugars), and may *only fortuitously give equivalent total weights*. Conversely, methods that give "different" fat values may actually have removed equivalent amounts of fat but different amounts of other material. Because fat extracts using various solvents, both with and without hydrolysis, may contain nonfats (McGhee *et al.*, 1974), they should be analyzed for their nonfat content. Therefore, when comparing fat methods, it is necessary to use an analytical technique specific for fatty acids (e.g. a GC-FAME) to determine the actual amount of fat present. If total fat is to be determined, a method that removes bound as well as free lipids should be used.

Importance of fat-specific analysis

The selective extraction of only the analyte of interest away from interfering components is relatively rare and, in general, the analyte of interest is coextracted with interfering compounds (King, 1989). SFE is not immune to wide variations in gravimetrically determined fat values either. Gravimetric fat values ranging from 10.7 to 19.6 weight percent were reported for a ground turkey sample, depending on the SFE extraction conditions, modifiers used, etc. (King, 1994). If the extraction method extracts both the analyte of interest and the interfering compounds, the relative amounts of these components must be determined before the true amount of analyte can be determined. In some cases, a chromatographic technique may be used to separate and quantify all extract components. However, in most cases, the components are not compatible on the same chromatographic system (e.g. sugars and fats by GC) and must be separated before analysis. Although mixtures containing components such as mono- and triglycerides cannot be analyzed by gas chromatography, complex mixtures containing nonpolar to moderately polar compounds with molecular weights between 100 to 1000 Da can be analyzed by SFC without derivatization (King, 1990). In our laboratory, we have found SFC an invaluable tool for the analysis of mixtures not easily analyzed in any other way. We routinely use SFC to analyze lipid mixes containing free fatty acids, FAMES, mono-, di-, and/or triglycerides. Sheppard *et al.* (1974), using a GC-FAME method, reported that for several food products, an HCl digestion followed by ether extraction was the most effective fat extraction method.

Problems with traditional organic solvent-based extractions

Current extraction methods, such as soxhlet, generally use large quantities of organic solvents (e.g. hexane, chloroform, or ether) (e.g. House *et al.*, 1994) and require evaporation of the solvent before subsequent gravimetric determination (Lumley and Colwell, 1991). In addition, soxhlet methods may not extract bound lipids and require long extraction times, and the amount of lipids extracted may be dependent on the solvent used, the drying method, and the fat content of the sample (Hagan *et al.*, 1967). These solvents are also potential hazards to both users and the environment.

SFE: importance/basis of the technique

Pertinent regulatory legislation

Because of their adverse environmental impact, the Environmental Protection Agency (EPA) has directed government agencies to reduce their consumption of organic solvents in Federal laboratories (Federal Register, 1993b). In addition, the costs of both purchase and disposal of these solvents has stimulated interest in alternative methods that use fewer organic solvents. Our research group has been investigating supercritical fluid extraction (SFE) as an alternative to solvent-based extraction methods.

Comparison with traditional methods

Supercritical fluids possess unique solvent properties, including diffusivities similar to gases and solvating strengths approaching those of liquids. In addition, there is the possibility to adjust the solvent power (and control the selectivity) of a supercritical fluid by adjusting the pressure and temperature (Stahl *et al.*, 1980). Although various kinds of supercritical fluids have been studied (Wilke, 1978), most work has been done on carbon dioxide (Yamaguchi *et al.*, 1986), and our discussion of the SFE of fat will be limited to supercritical carbon dioxide (SC-CO₂).

The use of SC-CO₂ has many advantages. Carbon dioxide is relatively nontoxic, is noncombustible, has a low critical temperature (31°C), and is cheap (Yamaguchi *et al.*, 1986). SFE methods employing SC-CO₂ are environmentally benign, can result in reduced extraction times, and can be automated (Lehotay *et al.*, 1995). In addition, SC-CO₂ can be removed easily from the extract (i.e. no solvent residue), and there are no costs associated with solvent waste disposal (Stahl *et al.*, 1980).

The decreased use of organic solvents associated with SC-CO₂ extractions can reduce the exposure of lab personnel to both the health- and safety-related problems of organic solvents. Fat recoveries for SFE and solvent-based extraction methods are in good agreement (Taylor *et al.*, 1993), and the precision of analytical SFE is comparable to that of traditional organic solvent-based methods, and in some cases is better (King *et al.*, 1996).

SFE: application of the technique

Apparatus required and instrumentation available

Because the equipment required to perform supercritical extractions is somewhat sophisticated, the initial costs of SFE equipment is generally higher than that for other "standard" extraction methods such as soxhlet. Although SFE equipment can be self-assembled relatively easily (our laboratory routinely uses home-built extractors to perform a variety of SFE experiments, e.g.

King *et al.*, 1995), most labs do not have the expertise or desire to fabricate such equipment and prefer to purchase SFEs from a manufacturer. Fortunately, a variety of commercial SFE units are available from several vendors. Generically speaking, an analytical supercritical fluid extractor consists of a fluid delivery module, a source of fluid (e.g. CO₂), an extraction cell, a back pressure regulating device, and a collector for trapping the analyte.

Fluid delivery module

There are a plethora of fluid delivery devices, including diaphragm compressors, gas or liquid booster pumps, reciprocating piston pumps, syringe pumps, and thermal pumps. Each device has its own merits and limitations, but some general principles are worth consideration. Most syringe pumps and plunger-based pumps require an external cooling source to assure liquefaction of the fluid and avoidance of cavitation at the pump head. One commercial unit uses dry CO₂ for cryogenic cooling (i.e. Joule-Thomson effect) of the pump heads. Although effective, this method is cumbersome, inconvenient, and somewhat costly. We find that the use of recirculating water baths is very effective and is the most convenient method of cooling the pump heads. Some manufacturers, to avoid cooling the pump heads, suggest the use of helium headspace CO₂ as a pressure pad in the gas cylinder. This design has several disadvantages, including the added cost of this type of CO₂, the decreased amount of CO₂ available, and the decreased solubility of some analytes (including lipid moieties) in the CO₂ pressurized with helium (King *et al.*, 1995). In addition, the solvation power of CO₂ from tanks pressurized with helium changes as the tank is emptied (Zhang and King, 1997, in press), and this should be taken into account when performing extractions with this type of CO₂.

Source of fluid

The purity of the CO₂ used for extraction can be very important to avoid the potential addition of interferences. Although CO₂ manufacturers do provide SFE grades (ca. 1 ppb nonvolatile hydrocarbon residue) of CO₂, these grades can be very expensive (Taylor, 1996) and are subject to availability and delivery schedules. Because of this, there is a real need for the development of sorbent-based gas purification systems to attach to extraction modules (King and Hopper, 1992). It should be noted that gravimetric-based SFE determinations do not require ultrapure CO₂.

Cosolvents (modifiers)

The need to use small quantities of organic solvents in many SFE procedures has become apparent as the technique has matured (King, 1993b). These cosolvents are generally used to increase the solubility of the analyte or possibly to increase the separation of coextractives (King and France, 1992). Cosolvents such as ethanol have been used to increase the solubility of phospholipids in SC-CO₂ (Temelli, 1992; Montanari *et al.*, 1996). Performing SFE with cosolvents usually results in a higher weight percent of fat over that recorded with pure CO₂.

Cosolvents can be added directly to the extraction cell, added to the CO₂ flow using a second pump, or added with the CO₂ from a premixed cylinder. When modifiers are added to the extraction cell, their effects are limited to the time they are present, and they end when they are removed by the CO₂. With premixed cylinders, the concentration of the modifier can change as the cylinder is emptied, having significant effects on its solvating power. Several commercial instruments have the capability of continuous addition of cosolvent during an extraction.

Extraction cell

All of the commercial extraction cells are designed to hold solid samples, although there are instances where the ability to extract a liquid sample would be useful. For quantitative extrac-

tions, large samples generally require longer extraction times. Therefore, smaller samples are preferred unless larger samples are required to insure sample homogeneity or to provide the required analytical sensitivity (Hawthorne, 1990). The maximum cell sizes compatible with commercially available equipment are somewhat limited, especially for the automated units, ranging from 0.5–10 mL. The small sample sizes that can be extracted can be a problem (King, 1993b), especially for samples with low levels of fat or those that may require the addition of some adsorbent, such as pelletized diatomaceous earth (Hopper and King, 1991). In cases where the sample alone does not fill the extraction cell, it is generally recommended that the void volume be filled with some inert material such as glass wool, glass beads, or Hydromatrix. This prevents channeling of the solvent through the cell and also reduces the volume of solvent required for the extraction. Care should also be taken not to overfill the extraction cell, which may also lead to incomplete extraction of the analyte.

Back pressure regulating device

Although back pressure control can be accomplished using either fixed restrictors or variable restrictors, commercial SFEs generally use variable flow restrictors. Restrictors on some commercial SFEs provide automated variable flow control, and some are heated to prevent clogging by ice or freezing of other solutes. The restrictor is the most frequent trouble spot of an SFE instrument, and low recoveries can often be attributed to clogged or partially blocked restrictors (McNally, 1996).

Collection

Optimization of the collection step may be the most important step in the development of a SFE method (Lehotay, 1997, in press). The various commercial SFEs have a variety of means of sample collection. SFE effluents may be collected by direct depressurization into an empty vial, into solvent, or the effluent may be collected on inert supports (e.g. glass beads) or on active sorbents (e.g. silica or bonded phases) and subsequently eluted with a liquid solvent (Hawthorne, 1990; Taylor, 1996). Although most commercial SFEs allow the collection of an extract free of solvent, some are designed such that the extract is deposited on a trap that must subsequently be rinsed with a liquid solvent. Two commercial SFE units are designed with this type of "trap." These trap systems may increase the likelihood of carry-over between samples and eliminating the possibility of a solvent-free extract. One system has a long, unheated, coiled PEEK (polyetheretherketone) transfer line from the trap to the collection vial, where saturated fat tends to solidify and plug this line, leading to the loss of analyte through the solvent rinse line or through the over-pressure relief valve. On some SFEs, it is also possible to control the temperature of the collection vial to improve the efficiency. One unit allows for the pressurized collection of analytes in the collection vial, and one allows the addition of solvent to the collection vial to replenish that lost with the expanded CO₂ flow. For one SFE unit, it is recommended that analyte be collected in vials packed with glass wool. Although this unit is designed for measuring fat gravimetrically, it would be very difficult to remove the fat for chromatographic analysis, if so desired. It has been our experience that direct depressurization into an empty test tube is generally sufficient for collecting extracted fat (King *et al.*, 1995).

Role of support analytical methodology

Supercritical extractions can be performed either "off-line" (i.e. separate from) of the subsequent analytical technique or "on-line" (i.e. directly connected to). On-line techniques eliminate the need to handle the sample between extraction and analysis (Hawthorne, 1990), how-

ever, because on-line techniques are in general more complex and difficult to implement and require the continual commitment of analysis equipment, most supercritical extractions are done off-line from the subsequent analytical method (Taylor, 1996). Examples of the on-line combination of SFE and high-performance liquid chromatography (HPLC) (Unger and Roumeliotis, 1983; Engelhardt and Gargus, 1988), capillary GC (Hawthorne and Miller, 1987) and (on-line) GC/MS (Snyder *et al.*, 1993), SFC-packed column (Sugiyama *et al.*, 1985; Engelhardt and Gross, 1988; McNally and Wheeler, 1988), SFC-capillary (Gmuer *et al.*, 1987a; King, 1990) have been reported. A variety of detectors have been used in SFC. Examples include ultraviolet (UV) (Sugiyama *et al.*, 1985), mass spectrometry (MS) (Smith *et al.*, 1984), Fourier transform infrared spectroscopy (FTIR) (Shafer and Griffiths, 1983; Yang *et al.*, 1991), and flame ionization detection (FID) (Rawdon, 1984). There is a commercially available SFE-IR for the analysis of fats (see the previous article in this issue).

Sample preparation/extraction/collection/reaction

Matrix effects: lipid distribution/association of fat with other components

Matrix characteristics that affect classical liquid-liquid or liquid-solid extraction also influence the efficiency of SFE (McNally, 1996). In SFE, as in most extraction procedures, the effects of the sample matrix are the least understood. Variability of matrix type and the physical and chemical complexity of matrices can make extractions difficult (McNally, 1995). Although the distribution of lipids within foods is often ignored, it can have significant effects on the extraction of the lipids from the foods (Fritsch, 1994). In low-fat foods, the lipids are generally in a noncontinuous lipid phase dispersed within polar components (e.g. polysaccharides or proteins), similar to an oil-in-water emulsion (Fritsch, 1994). These polar compounds may form a barrier and may preclude the complete extraction of lipids, because the lipids must be transported across this layer before they can be removed by the solvent. In bread dough, the gluten proteins form a lipoprotein complex that is impermeable to some solvents and precludes complete extraction of lipids (Finney *et al.*, 1976). However, in high-fat foods, the lipid phase is usually continuous with polar components dispersed within the fat. In these cases, the solvent has direct contact with the lipids, making their subsequent extraction easier.

Although SC-CO₂ is more effective at extracting bound lipids than hexane (Christianson *et al.*, 1984), it does not remove them completely either. As with organic solvent extractions, to determine "total lipid" a digestion step is often required to release bound lipids. Because phospholipids are insoluble in SC-CO₂ (Friedrich *et al.*, 1982; Yamaguchi *et al.*, 1986; Fattori *et al.*, 1987), phospholipids (and the fatty acids they contain) are absent in these extracts and subsequently are not available for measurement of "total" fat as defined by NLEA. Although this is a potential benefit to oil producers (i.e. the oils do not need to be degummed) and it may be an effective means of separating these components for separate analyses, they are not measured together as total fat. Lipids of aquatic organisms are high in phospholipids (Yamaguchi *et al.*, 1986), and these would be absent in a SC-CO₂ extract without a prior hydrolysis step or suitable modifier.

Comminution of the sample

The apparent fat content can be affected by the particle size extracted. Both Finney *et al.* (1976) and McGhee *et al.* (1974), using organic solvents, reported improved fat extraction with smaller particle sizes, presumably a result of the more intimate contact of the solvent with the lipid in the sample. Similarly, Stahl *et al.* (1980), using supercritical CO₂, reported that fats were most

easily removed from small particles. In addition, King *et al.*, (1989) reported that triglycerides were more rapidly extracted from ground samples than unground samples, and Snyder *et al.* (1984) reported that the supercritical extraction of oilseeds was much higher for ground and flaked seeds than for cracked seeds.

Effect of water content

Although the addition of water improves the supercritical extraction of caffeine from coffee (Zosel, 1980), the presence of water seems to have little effect on the SFE of oils (Snyder *et al.*, 1984; Christianson *et al.*, 1984). For nonpolar analytes (e.g. triglycerides), the presence of water is generally detrimental (McNally, 1995). Hopper and King (1991) state that samples containing more than 10% water can interfere with the SFE of lipids from many sample types. King *et al.* (1989; King, 1994) reported that fats were more effectively extracted from dried meat samples than wet samples. Snyder *et al.* (1997, in press) found that water inhibited the enzymatic transesterification of fats using SFE-SFR. It seems that a partial dehydration of the sample matrix allows a more rapid and complete SFE to be performed. This is due to the fact that hydrophilic matrices inhibit contact between the supercritical fluid and the target analyte (King and France, 1992).

In addition to its potential negative effects on lipid extraction, water is somewhat soluble in SC-CO₂ (Evelein *et al.*, 1976), and this may give somewhat inflated gravimetric fat determinations due to this coextracted water. Taylor *et al.* (1993) gives a graph of the solubility of water in CO₂ for various temperature-pressure combinations. It is recommended that collected samples be given a quick rotary evaporation (Taylor *et al.*, 1993) or that the collected oil be heated, either with sparging or rotary evaporation (King *et al.*, 1993a) to remove coextracted water.

King *et al.* (1993a) found that the weight loss of material in the extractor exceeded the weight of material collected. This was due to extraction of water from the matrix. Because of the solubility of water in CO₂, it is not recommended that fat content be estimated based on the difference between weight before and after SFE.

Control of water during SFE

Although it is possible to remove moisture from samples by freeze drying, this method is somewhat expensive, time consuming, and may cause the loss of volatile analytes (Hopper and King, 1991). If heat is used to dry the sample before extraction, the heat may cause the loss of the more volatile lower chain fatty acids or cause air oxidation of unsaturated components (Carpenter *et al.*, 1993), or possibly may cause the polymerization of oils (Firestone, 1963). For this reason, dehydrating sorbents or extraction enhancers have been developed for use with SFE. Hopper and King (1991) reported the enhanced SFE of a variety of foods by mixing the matrix with Hydromatrix (Celite 566) (i.e. pelletized diatomaceous earth) before extraction. This patented process (Hopper and King, 1992), makes a free-flowing mixture, and improved SFE is thought to be a result of the absorption of water, the dispersement of the sample, and the reduction of channeling through the extraction cell (Hopper and King, 1991). However, depending on the percentage of water in the sample, such enhancers can limit the amount of actual sample that can be added to the extraction cell (Hopper and King, 1991). Other materials that have been used to dehydrate the sample include sodium sulfate and calcium sulfate.

Sample fractionation in SFE

Adsorption as a complimentary process to SFE confers an extra degree of flexibility in segregating and fractionating solutes dissolved in the fluid phase (King *et al.*, 1988). King (1987)

discusses the use of adsorbents such as activated carbon, ion exchange resins, alumina, and porous polymers. Selectivity can be enhanced for an analyte by incorporating adsorbents *in situ* or after the initial extraction (King and Hopper, 1992). The use of sorbent-based technologies, either integrated into the SFE step or used after SFE appear to be the most promising cleanup technologies (King, 1993b). King (1989) discusses possible means such as the use of sorbents to retain and/or separate analytes of interest from interferences.

Although it is generally desirable to extract the analyte of interest from the matrix, leaving behind interfering compounds as well, it is possible to extract the unwanted interfering compounds, leaving behind the analyte of interest, a form of "inverse SFE" (King and Hopper, 1992). This type of sample cleanup has already been demonstrated on an engineering scale, and it offers the possibility of isolating analytes from interfering components (King and Hopper, 1992).

Carbon dioxide is soluble in triglycerides (Brunner and Peter, 1982) and may be imbibed in the collected oil and fat after SFE. Hence, this dissolved CO₂ can give high gravimetric fat values if not removed (e.g. by rotary evaporation) from the oil before being weighed (Taylor *et al.*, 1993).

Optimum extraction conditions: solubility of lipids in SC-CO₂

In general, the solubility of triglycerides increases with both extraction temperature and pressure, although there is an inversion in the solubility-temperature relationships as a function of pressure (Stahl *et al.*, 1980; Friedrich *et al.*, 1982; DeFilippi, 1982) (Figure 1). Because the solubility of triglycerides increases greatly with pressure, supercritical fluid extractions of fat are most efficiently done at high pressures. An example of the importance of extraction pressure and temperature on the quantity of fluid required for SFE is shown in Figure 2, where the weight percent solubility of triglycerides is plotted versus the mass of CO₂ used. Here the obvious benefit of extracting at 12,000 psi and 80°C versus 8,000 psi and 50°C is apparent, because the quantity of CO₂ required and the time to complete the extraction is minimized at a higher pressure and temperature. Although some SFEs have a high maximum operating pressure (e.g. 9,500 or 10,000 psi), others have much lower maximum pressure capabilities (e.g. 5,500 or 6,000 psi), limiting the achievement of the maximum solubility of triglycerides in SC-CO₂.

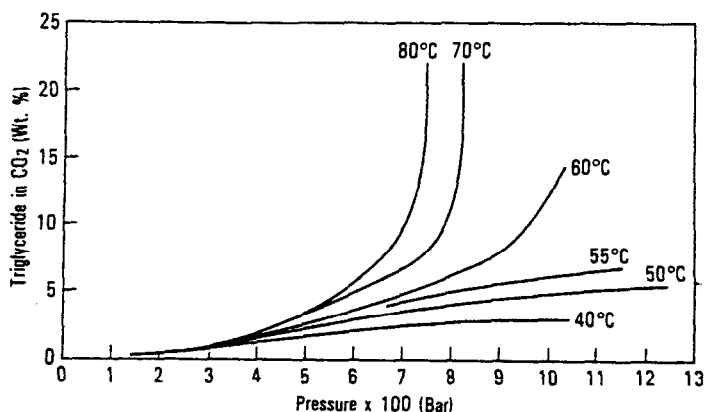


Fig. 1. Solubility of soybean oil triglycerides in supercritical CO₂ as a function of pressure and temperature.

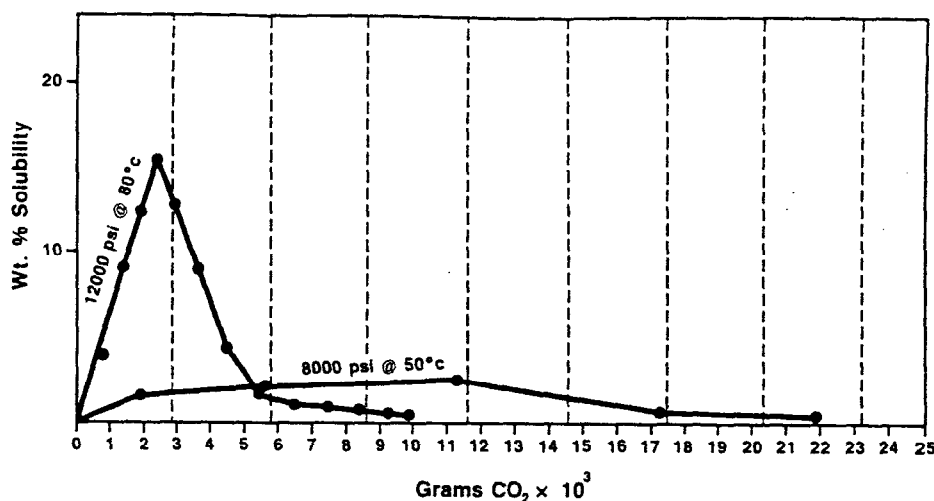


Fig. 2. Solubility of cottonseed oil triglycerides versus mass of CO₂ passed through seed bed.

It should be noted that there can be some discrimination with respect to fatty acid chain length during the SFE of fatty acids. The solubility of C₂₂ and C₂₄ fatty acids was less than that of shorter chain fatty acids during their extraction from canola (Fattori *et al.*, 1987). The later fractions contained a higher percentage of C₂₂ and C₂₄ fatty acids than the earlier fractions, which would lead to an incorrect determination of the fatty acid profile. Although this may be minimal during the SFE of FAME derivatives, it is very important to determine the length of extraction required to quantitatively remove all of the target analyte. Using some of the highly automated commercial SFE equipment, method optimization studies can be conducted very easily and quickly.

Optimizing collection conditions

In general, the quantitative collection of analytes is easier at lower flow rates (Hawthorne, 1990). However, there is a trade-off between collection efficiency and speed of extraction, and an appropriate compromise may be required.

Method development and optimization can be performed rapidly and relatively easily with automatic SFEs, which can be used to compare an array of parameters such as extraction temperature, pressure, static extraction time, dynamic extraction time, flow rate, restrictor temperature, collector temperature, modifier addition, and pressurized collectors (McNally, 1996).

Supercritical fluid reaction

A sequential supercritical fluid extraction-supercritical fluid reaction (SFE-SFR) method using enzymatic transesterification has been described for GC-FAME analysis of meat (Snyder *et al.*, 1996) and oilseeds (Snyder *et al.*, 1997). This one-step extraction/transesterification is a very efficient and convenient method for the analysis of fats according to NLEA requirements. An example of the results obtained by this method and their comparison to results determined using conventional solvent extraction are noted in Table 1 for various meat samples (Snyder *et al.*, 1996).

Table 1. Comparison of Total Fat Results from SFE/SFR Method and Conventional Solvent Extraction Method

Sample	Weight percent of total fat (RSD) ^a	
	SFE/SFR	Solvent extraction
Bacon	39.4 (3.4)	38.7 (1.7)
Beef (low fat)	11.2 (5.5)	12.8 (5.6)
Beef (medium fat)	20.6 (2.3)	21.8 (4.5)
Beef (high fat)	28.8 (2.3)	28.6 (3.7)
Ham (low fat)	9.9 (5.5)	10.0 (3.)
Ham (high fat)	16.5 (4.1)	17.1 (0.8)
Sausage (low fat)	11.1 (6.8)	9.1 (2.3)
Sausage (medium fat)	15.8 (3.7)	13.9 (3.3)
Sausage (high fat)	20.6 (6.1)	20.3 (2.2)

^a(RSD) = Relative Standard Deviation, $n = 3$.**Examples of SFE of foods for fat assay***Oilseeds*

Triglycerides have been extracted from soybeans (Stahl *et al.*, 1980; Friedrich *et al.*, 1982; Snyder *et al.*, 1984; List *et al.*, 1989; Taylor *et al.*, 1993), canola (Stahl *et al.*, 1980; Friedrich *et al.*, 1982; Fattori *et al.*, 1987; Taylor *et al.*, 1993), corn (List *et al.*, 1989; Taylor *et al.*, 1993; Christianson *et al.*, 1984), cottonseed (List *et al.*, 1989; Snyder *et al.*, 1984), peanuts (Snyder *et al.*, 1984), and coconut (Brannolte *et al.*, 1983). An excellent example of analytical SFE for three different oilseed types is provided by Taylor *et al.*, (1993) in Table 2. Here it can be seen that the SFE results agree well with those obtained by an "official" Soxhlet method using gravimetry to determine the final oil yield.

Meat

Although there have been a fair number of studies using SFE to extract fat from plant products, applications of SFE to animal sources are somewhat more limited. Yamaguchi *et al.* (1986) and Hardardottir and Kinsella (1988) extracted triglycerides from dehydrated fish muscle. King *et al.* (1989) reported the efficient SFE of fats from meat samples ranging in fat content from 2–35%. Chao *et al.* (1991) extracted triglycerides from ground beef. Lembke and Engelhardt (1993) conducted an SFE of fats retained on filter paper from meat hydrolysate. Sawyer (1993)

Table 2. Analytical-Scale Extraction of Various Seed Oil Commodities: SFE versus Soxhlet

Sample	Weight percent of recovery (\pm SD)	
	SFE ^a	Soxhlet ^b
Soybean flakes	20.6 (\pm 0.2)	20.5 (\pm 0.2)
Canola	39.8 (\pm 0.5)	40.5 (\pm 0.5)
Wet-milled corn germ	48.9 (\pm 0.5)	50.4 (\pm 1.3)

^a $n = 4$.^b $n = 5$.

reported the efficient SFE of fat from pork sausage meat. For ground beef samples, King (1994) and King *et al.* (1996) reported that gravimetrically determined SFE fat values and GC-FAME fat values, respectively, were nearly identical to those determined by solvent extraction and GC-FAME analysis.

Dairy products

SFE has been used to extract fat from butter (Kaufmann *et al.*, 1982; Gmuer *et al.*, 1987b), cheese (Gmuer *et al.*, 1987b; Sawyer 1993), and milk (Arul *et al.*, 1987). Froning *et al.*, (1990) used SFE to extract lipids from eggs.

Snack foods

Hopper and King (1991) report the extraction of fat from peanut butter as well as other foods, and Sawyer (1993) reported the extraction of fat from corn chips. An example of SFE for this application is shown in Table 3. These were obtained from extractions performed in our laboratory on snack foods where fat content ranged from 2–50 weight percent.

Cereals and baked goods

For several types of bakery goods, Bowadt (unpublished manuscript) has found excellent agreement between an SFE method using acid hydrolysis and an ethanol modifier to results obtained using acid hydrolysis and standard organic solvents (Gelroth, unpublished manuscript).

Potential application to other lipid moieties

Other lipids, such as sterols, are soluble in SC-CO₂ (King, 1983; Chrastil, 1982). Although not soluble in SC-CO₂ alone (Friedrich *et al.*, 1982), phospholipids can be solubilized easily by the addition of modifiers, such as ethanol (Temelli, 1992; Montanari *et al.*, 1996). Chao *et al.* (1991) reported the SC-CO₂ extraction of cholesterol from ground beef. Bradley (1989) and Ong *et al.* (1990) reported the SFE of cholesterol from milk fat and eggs, respectively. Artz and Myers (1995) have also reported the SFE of acetylated monoglycerides.

Future prospectus

Can industry meet the challenge?

Although SFE was first demonstrated to be a highly accurate and reproducible technique on noncommercial instruments, SFE methods will never be adopted for routine use unless they can

Table 3. Weight Percent Fat in Snack Food Products as Determined by Analytical Supercritical Fluid Extraction

Sample	Weight percent recovery
Fat-free oatmeal raisin cookies	1.78
Corn chips	19.6
Potato chips	22.6
M & M peanut chocolates	24.7
Frosted butter cookies	25.9
Cheese curls	32.8
Cheddar cheese	33.3
Ripple style potato chips	34.5
Peanut butter	49.5

be translated to commercial models (King *et al.*, 1993b). Analytical SFE is currently undergoing a transition from laboratories conducting basic research to those conducting method development as well as the analysis of real samples (McNally, 1995). There are currently several manufacturers of commercial SFEs available, and each extractor has its own specific strengths and weaknesses. The most versatile SFE in terms of varied sample size is a single sample unit employing a manual restrictor. The available automated units are the least versatile in terms of sample size and collection methods (see Lehotay, 1997, in press). One commercial unit that does parallel (batch) extractions, can handle up to nine samples at once (requiring 3 extractors with 3 extraction cells each) and can give replicate analyses very quickly. Because of these differences in equipment and user needs, specific commercial instruments may be better suited to a specific method (King *et al.*, 1993b).

Can automation meet the challenge?

Laboratories that routinely analyze large numbers of samples, (e.g. quality control or regulatory labs) have a need for automated systems, and the need for such equipment that can automatically process large numbers of samples has been recognized for some time (King, 1993b). Manufacturers have moved quickly to develop commercial SFE instruments capable of the analysis of multiple samples either in a parallel (batch) or serial mode. The recent introduction of automated commercial SFEs capable of extracting large numbers of samples (i.e. up to 24 or 44) sequentially was required by labs needing to conduct analyses on large numbers of samples.

The availability of such commercial instrumentation that can be applied to a wide variety of sample matrices and analytical problems should accelerate the growth of SFE techniques (King *et al.*, 1993b). Although these recent instrument developments portend a promising future for SFE analysis of fat (King and France, 1992), the recent exodus of several companies from this field may limit the options available to the analyst. On the other hand, interesting opportunities exist for companies that can address the future needs of the field, particularly instrumentation that can be used for both SFE and combinations with liquid extractions.

Collaborative studies to date

Although analytical SFE is a promising technique and instrumentation is available, acceptance of an SFE method as a standard requires collaborative studies to verify its reproducibility (King, 1992; King, 1993a). Although several "round-robin" studies have been initiated by several agencies (e.g. EPA, NIST, USDA) (King *et al.*, 1993b), to date only one SFE-based method has received approval by AOAC/AACC/AOCS for determining the fat content in oilseeds (no. Am 3-96). The validation of any method, including SFE, requires a great deal of time, effort, and expense (Lehotay, 1997, in press), and our laboratory has experienced the difficulties associated with undertaking such studies. However, if SFE is to remain a viable technique, more effort needs to be directed toward collaborative studies.

Although no single technique can solve the diversity of problems confronting the analytical chemist, SFE has a rightful place among other sample preparation methods. Although legislated reductions in solvent use may speed the implementation/growth/development/acceptance of SFE, successful implementation and widespread acceptance of SFE will require that analysts expand their horizons and trade in some of their conventional tools such as soxhlet extractors, separatory funnels for extraction cells, and pressure monitors (King and Hopper, 1992). In addition, advocates/proponents of SFE must continue to integrate SFE into established protocols, thereby facilitating the transition of the technology to the analytical chemist (King and Hopper, 1992). In addition, industrial and governmental laboratories must make their needs

known to instrument manufacturers if the technique is to remain viable (King and Hopper, 1992). And finally, there needs to be increased communication, cooperation, and collaboration between SFE vendors and analytical chemists to achieve the successful integration of this promising technique into the repertoire of the analyst.

References

- Artz, WE, Myers, MR. (1995) Supercritical fluid extraction and chromatography of emulsifiers. *J Am Oil Chem Soc* **72**, 219–224.
- Arul, J, Boudreau, A, Makhoul, J, Tardif, R, Sahasrabudhe, MR. (1987) Fractionation of anhydrous milk fat by supercritical carbon dioxide. *J Food Sci* **52**, 1231–1236.
- Bradley, RL, Jr. (1989) Removal of cholesterol from milk fat using supercritical carbon dioxide. *J Dairy Sci* **72**, 2834–2840.
- Brannolte, H, Mangold, HK, Stahl, E. (1983) Effects of pressure and temperature of supercritical carbon dioxide on the extraction of triacylglycerols from plant tissue. *Chem Phys Lipids* **33**, 297–299.
- Brunner, G, Peter, S. (1982) On the solubility of glycerides and fatty acids in compressed gases in the presence of an entrainer. *Sep Sci* **17**, 199–214.
- Carpenter, DE, Ngeh-Ngwainbi, J, Lee, S. (1993) Lipid analysis. In *Methods of analysis for nutrition labeling* (DM Sullivan and DE Carpenter, eds.). Chapter 5. AOAC International, Arlington, VA.
- Chao, RR, Mulvaney, SJ, Bailey, ME, Fernando, LN. (1991) Supercritical CO₂ conditions affecting extraction of lipid and cholesterol from ground beef. *J Food Sci* **56**, 183–187.
- Chrastil, J. (1982) Solubility of solids and liquids in supercritical gases. *J Phys Chem* **86**, 3016–3021.
- Christianson, DD, Friedrich, JP, List, GR, Warner, K, Bagley, AC, Stringfellow, AC, Inglett, GE. (1984) Supercritical fluid extraction of dry-milled corn germ with carbon dioxide. *J Food Sci* **49**, 229–233.
- DeFilippi, RP. (1982) CO₂ as a solvent: application to fats, oils and other materials. *Chem Ind June* **19**, 390–393.
- Engelhardt, H, Gross, A. (1988) On-line extraction and separation by supercritical fluid chromatography with packed columns. *J High Resolut Chromatogr Commun* **11**, 38–42.
- Engelhardt, WG, Gargus, AG. (1988) Supercritical fluid extraction for automated sample preparation. *Am Lab* **20**, 30–32.
- Evelein, KA, Moore, RG, Heidemann, RA. (1976) Correlation of the phase behavior in the systems hydrogen sulfide-water and carbon dioxide-water. *Ind Eng Chem Process Dev* **15**, 423–428.
- Fattori, M, Bulley, NR, Meisen, AJ. (1987) Fatty acid and phosphorous contents of canola seed extracts obtained with supercritical carbon dioxide. *J Agric Food Chem* **35**, 739–743.
- Federal Register (1993a) **58**, 631–2964.
- Federal Register (1993b) **58**, 41983.
- Finney, KF, Pomeranz, Y, Hoseney, RC. (1976) Effects of solvent extraction on lipid composition, mixing time, and bread loaf volume. *Cereal Chem* **53**, 383–338.
- Firestone, D. (1963) The determination of polymers in fats and oils. *J Am Oil Chem Soc* **40**, 247–255.
- Friedrich, JP, List, GR, Heakin, AJ. (1982) Petroleum-free extraction of oil from soybeans with supercritical CO₂. *J Am Oil Chem Soc* **59**, 288–292.
- Fritsch, CW. (1994) Lipid oxidation-the other dimensions. *INFORM* **5**, 423–432.
- Froning, GW, Wehling, RL, Cuppett, SL, Pierce, MM, Nieman, L, Siekman, DK. (1990) Extraction of cholesterol and other lipid from dried egg yolk using supercritical carbon dioxide. *J Food Sci* **55**, 95–98.
- Gmuer, W, Bosset, JO, Plattner, E. (1987a) Contribution to direct coupling of supercritical fluid extraction to capillary supercritical fluid chromatography. I. Theoretical optimization of some important instrumental parameters. *J Chromatogr* **388**, 143–150.
- Gmuer, W, Bosset, JO, Plattner, E. (1987b) Direct coupling of supercritical fluid extraction to capillary

- supercritical fluid chromatography. II. Construction of a prototype and examples of application. *J Chromatogr* **388**, 335–349.
- Hagan, SN, Murphy, EW, Shelly, LM. (1967) Extraction of lipids from raw beef lean by using various solvent systems. *J Assoc Off Anal Chem* **50**, 250–255.
- Hardardottir, I, Kinsella, JE. (1988) Extraction of lipid and cholesterol from fish muscle with supercritical fluids. *J Food Sci* **53**, 1656–1658.
- Hawthorne, SB. (1990) Analytical-scale supercritical fluid extraction. *Anal Chem* **62**, 633A–642A.
- Hawthorne, SB, Miller, DJ. (1987) Directly coupled supercritical fluid extraction- gas chromatographic analysis of polycyclic aromatic hydrocarbons and polychlorinated biphenyls from environmental solids. *J Chromatogr* **403**, 63–76.
- Hertwig, R. (1923) Determination of fat in alimentary paste, flour and dried egg. *J Assoc Off Agric Chem* **6**, 508–510.
- Hopper, ML, King, JW. (1991) Enhanced supercritical fluid carbon dioxide extraction of pesticides from foods using pelletized diatomaceous earth. *J Assoc Off Anal Chem* **74**, 661–666.
- Hopper, ML, King, JW. (1992) Supercritical fluid extraction enhancer. United States Patent No. 5,151,188.
- House, SD, Larson, PA, Johnson, RR, DeVries, JW, Martin, DL. (1994) Gas chromatographic determination of total fat extracted from food samples using hydrolysis in the presence of antioxidant. *J Assoc Off Anal Chem* **77**, 960–965.
- Hubbard, WD, Shepard, AJ, Newkirk, DR, Prosser, AR, Osgood, T. (1977) Comparison of various methods for the extraction of total lipids, fatty acids, cholesterol, and other sterols from food products. *J Am Oil Chem Soc* **54**, 81–83.
- Inkpen, JA, Quackenbush, FW. (1969) Extractable and “bound” fatty acids in wheat and wheat products. *Cereal Chem* **46**, 580–587.
- Kaufmann, W, Biernoth, G, Frade, E, Merk, E, Precht, D, Timmen, H. (1982) Fraktionierung von butterfett durch extraktion mit überkritischem CO₂. *Milchwissenschaft* **37**, 92–96.
- King, JW. (1983) Generalized extraction conditions for the critical fluid processing of oils and oleophilic compounds. *J Am Oil Chem Soc* **60**, 711.
- King, JW. (1987) Supercritical fluid adsorption at the gas-solid interphase. In *Supercritical fluids: chemical and engineering principles and applications* (TG Squires and ME Paulaitis, eds.) Chapter 13. ACS Symp. Ser. 329.
- King, JW. (1989) Fundamentals and applications of supercritical fluid extraction in chromatographic science. *J Chromatogr Sci* **27**, 355–364.
- King, JW. (1990) Applications of capillary supercritical fluid chromatography-supercritical fluid extraction to natural products. *J Chromatogr Sci* **28**, 9–14.
- King, JW. (1992) Evolution and current status of analytical supercritical fluid techniques. In *Abstracts of the 4th international symposium on supercritical fluid extraction and chromatography*, Cincinnati, Ohio, May 20–22, 1992, pp. 237–239.
- King, JW. (1993a) Analysis of fats and oils by SFE and SFC. *INFORM* **4**, 1089–1096.
- King, JW. (1993b) Hazardous waste reduction in the development of analytical methods-Supercritical fluid Extraction. In *Symposium on new and emerging approaches to the development of methods in trace-level analytical chemistry*, Berkeley Springs, WV, August, 1992.
- King, JW. (1994) Analytical SFE applied in nutritional labeling analysis. In *Proceedings of the 3rd international symposium on supercritical fluids*, Strasbourg, France, Oct. 1994, Vol. 3, pp. 459–464.
- King, JW, Eller, FJ, Snyder, JM, Johnson, JH, McKeith, FK, Stites, CR. (1996) Extraction of fat from ground beef for nutrient analysis using analytical supercritical fluid extraction. *J Agric Food Chem* **44**, 2700–2704.
- King, JW, Eissler, RL, Friedrich, JP. (1988) Supercritical fluid-adsorbate-adsorbent systems. In *Supercritical fluid extraction and chromatography: techniques and applications* (BA Charpentier and MR Sevenants, eds.) ACS Symp. Ser. No. 366.
- King, JW, France, JE. (1992) Basic principles of analytical supercritical fluid extraction. In *Analysis with supercritical fluids* (B. Wencławiak, ed.) Chapter 3. Springer-Verlag, Berlin.

- King, JW, Hopper, ML. (1992) Analytical supercritical fluid extraction: current trends and future vistas. *J Assoc Off Anal Chem* **75**, 375–378.
- King, JW, Johnson, JH, Eller, FJ. (1995) Effect of supercritical carbon dioxide pressurized with helium on solute solubility during supercritical fluid extraction. *Anal Chem* **67**, 2288–2291.
- King, JW, Johnson, JH, Friedrich, JP. (1989) Extraction of fat tissue from meat products with supercritical carbon dioxide. *J Agric Food Chem* **37**, 951–954.
- King, JW, Johnson, JH, Orton, WL, McKeith, FK, O'Connor, PL, Novakofski, J, Carr, TR. (1993a) Fat and cholesterol content of beef patties as affected by supercritical CO₂ extraction. *J Food Sci* **58**, 950–952.
- King, JW, Snyder, JM, Taylor, SL, Johnson, JH. (1993b) Translation and optimization of supercritical fluid extraction methods to commercial instrumentation. *J Chromatogr Sci* **31**, 1–5.
- Lehotay, SJ. (1997) Supercritical fluid extraction of pesticide residues in fruits and vegetables. *Sem Food Anal* (in press).
- Lehotay, SJ, Aharonson, N, Pfeil, E, Ibrahim, A. (1995) Development of a sample preparation technique for multiresidue analysis of pesticides in produce. *J Assoc Off Anal Chem* **78**, 831–840.
- Lembke, P, Engelhardt, H. (1993) Development of a new SFE method for rapid determination of total fat content of food. *Chromatographia* **35**, 509–516.
- Lepper, HA, Watermann, HC. (1925) A method for the rapid and accurate determination of fat in cacao products. *J Assoc Off Agric Chem* **8**, 705–710.
- List, GR, Friedrich, JP, King, JW. (1989) Supercritical CO₂ extraction and processing of oilseeds. *Oil Mill Gaz* **95(6)**, 28–34.
- Lumley, ID, Colwell, RK. (1991) Extraction of fats from fatty foods and determination of fat content. In *Analysis of oilseeds, fats and fatty foods* (JB Rossell and JLR Pritchard, eds.) Chapter 6. Elsevier Applied Science, New York.
- Maxwell, RJ. (1987) Determination of total lipid and lipid subclasses in meat and meat products. *J Assoc Off Anal Chem* **70**, 74–77.
- McGhee, JE, Black, LT, Brekke, OL. (1974) Fat content in corn grits: effects of grinding, extraction solvents, and analytical methods. *Cereal Chem* **51**, 472–477.
- McNally, MEP. (1995) Advances in environmental SFE. *Anal Chem* **67**, 308A–315A.
- McNally, MEP. (1996) Method development in supercritical fluid extraction. *J Assoc Off Anal Chem* **79**, 380–387.
- McNally, MEP, Wheeler, JR. (1988) Supercritical fluid extraction couples with supercritical fluid chromatography for the separation sulfonylurea herbicides and their metabolites from complex matrices. *J Chromatogr* **435**, 63–71.
- Montanari, L, King, JW, List, GR, Rennick, KA. (1996) Selective extraction of phospholipid mixtures by supercritical CO₂ and cosolvents. *J Food Sci* **61**, 1230–1234.
- Ong, CP, Lee, HK, Li, FY. (1990) Supercritical fluid extraction and chromatography of cholesterol in food samples. *J Chromatogr* **515**, 509–513.
- Rawdon, MG. (1984) Modified flame ionization detector for supercritical fluid chromatography. *Anal Chem* **56**, 831–832.
- Sawyer, LD. (1993) Multiresidue methods. *J Assoc Off Anal Chem* **76**, 144–147.
- Shafer, KH, Griffiths, PR. (1983) On-line supercritical fluid chromatography/Fourier transform, infrared spectrometry. *Anal Chem* **55**, 1939–1942.
- Sheppard, AJ, Hubbard, WD, Prosser, AR. (1974) Evaluation of eight extraction methods and their effects upon total fat and gas liquid chromatographic fatty acid composition analyses of food products. *J Am Oil Chem Soc* **51**, 416–418.
- Smith, RD, Kalinoski, HT, Udseth, HR, Wright, BW. (1984) Rapid and efficient capillary column supercritical fluid chromatography with mass spectrometric detection. *Anal Chem* **56**, 2476–2480.
- Snyder, JM, Friedrich, JP, Christianson, DD. (1984) Effect of moisture and particle size on the extractability of oils from seeds with supercritical CO₂. *J Assoc Off Anal Chem* **61**, 1851–1856.

- Snyder, JM. King, JW. Jackson, MA. (1996) Fat content for nutritional labeling by supercritical fluid extraction and on-line lipase catalyzed reaction. *J Chromatogr A* **750**, 201–207.
- Snyder, JM. King, JW. Jackson, MA. (1997) Analytical supercritical fluid extraction using lipase-catalysis: conversion of different lipids to methyl esters and effect of moisture. *J Am Oil Chem Soc* (in press).
- Snyder, JM. Taylor, SL. King, JW. (1993) Analysis of tocopherols by capillary supercritical fluid chromatography and mass spectrometry. *J Am Oil Chem Soc* **70**, 349–354.
- Stahl, E. Schutz, E. Mangold, HM. (1980) Extraction of seed oils with liquid and supercritical carbon dioxide. *J Agric Food Chem* **28**, 1153–1157.
- Sugiyama, K. Saito, M. Hondo, T. Senda, M. (1985) New-double stage separation analysis method. *J Chromatogr* **332**, 107–116.
- Swern, D. (1964) Structure and composition of fats and oils. In *Bailey's industrial oil and fat products* (D. Swern, ed.) Chapter 1. Interscience Publishers, New York.
- Taylor, LT. (1996) *Supercritical fluid extraction*. John Wiley and Sons, New York.
- Taylor, SL. King, JW. List, GR. (1993) Determination of oil content in oilseeds by analytical supercritical fluid extraction. *J Am Oil Chem Soc* **70**, 437–439.
- Temelli, FJ. (1992) Extraction of triglycerides and phospholipids from canola with supercritical carbon dioxide and ethanol. *J Food Sci* **57**, 440–443.
- Unger, KK. Roumeliotis, P. (1983) On-line high-pressure extraction-high-performance liquid chromatography I. Equipment design and operation variables. *J Chromatogr* **282**, 519–526.
- Yamaguchi, K. Murakami, M. Nakano, H. Konosu, S. Kokura, T. Yamatoto, H. Kosaka, M. Hata, K. (1986) Supercritical carbon dioxide extraction of oils from Antarctic krill. *J Agric Food Chem* **34**, 904–907.
- Yang, J. Bonanno, AS. Norton, KL. Griffiths, PR. (1991) SFE/SFC of polar compounds from polar matrices with on-line FT-IR discrimination. *Abstracts of the 3rd International Symposium on Supercritical Fluid Chromatography and Extraction*, Park City, Utah.
- Wilke, G. (1978) Extraction with supercritical gases—a foreword. *Angew Chem Int Ed Engl* **17**, 701.
- Zhang, Z. King JW. (1997) The effects of dissolved helium on the density and solvation power of supercritical carbon dioxide. *J Chromatogr Sci* (in press).
- Zosel, K. (1980) Separation with supercritical gases: practical applications. In *Extraction with supercritical gases* (GM Schneider, E Stahl, and G Wilke, eds.) pp. 1–23. Verlag Chemie, Weinheim, Germany.

SFE BIBLIOGRAPHY

The information provided in this document is a bibliography of SFE references from 1990–1995. The list is not meant to be an exhaustive compilation but to provide you with a initial list for further investigations.

- King, JW. (1995) Determination of the solubility parameter of soybean oil by inverse gas chromatography. *Lebensm Wiss Technol* **28**(2), 190–195.
- Magard, MA, Berg, HEB, Tagesson, V, Jaeremo, MLG, Karlsson, LLH, Mathiasson, LJE, Bonneau-M. Hansen-Moller, J. (1995) Determination of androstenone in pig fat using supercritical fluid extraction and gas chromatography-mass spectrometry. *J Agric Food Chem* **43**(1), 114–120.
- Blanch, GP, Reglero, G, Herraiz, M. (1995) Analysis of wine aroma by off-line and on-line supercritical fluid extraction-gas chromatography. *J Agric Food Chem* **43**(5), 1251–1258.
- Nielsen, T. (1995) Aroma sorption by food packaging polymers studied with SFE-GC and other methods. *Diss Abstr Int C* **56**(1), 72–73.
- Paquet, A, Khan, SU. (1995) Release of covalently bound metabolites of organophosphate pesticides from synthetic dialkyl phosphoserine peptides by supercritical fluid extraction. *J Agric Food Chem* **43**(3), 843–848.
- Lembke, P, Boernert, J, Engelhardt, H. (1995) Characterization of irradiated food by SFE and GC-MSD. *J Agric Food Chem* **43**(1), 38–45.
- Lancas, FM, Queiroz, MEC, Silva, ICE. (1995) Simplex optimization of extraction of soybean oil by supercritical pentane. *Chromatographia* **40**(7/8), 421–424.
- Artz, WE, Myers, MR. (1995) Supercritical fluid extraction and chromatography of emulsifiers. *J Am Oil Chem Soc* **72**(2), 219–224.
- Dugo, P, Mondello, L, Bartle, KD, Clifford, AA, Breen, DGPA, Dugo, G. (1995) Deterpenation of sweet orange and lemon essential oils with supercritical carbon dioxide using silica gel as an adsorbent. *Flavour Fragrance J* **10**(1), 51–58.
- Aharonson, N, Lehotay, SJ, Ibrahim, MA. (1994) Supercritical fluid extraction and HPLC analysis of benzimidazole fungicides in potato, apple, and banana. *J Agric Food Chem* **42**(12), 2817–2823.
- Buecherl, T, Gruner, A, Palibroda, N. (1994) Rapid analysis of polymer homologues and additives with SFE/SFC-MS coupling. *Packaging Technol Sci* **7**(3), 139–153.
- Johansen, HR, Becher, G, Greibrokk, T. (1994) Determination of planar PCBs by combining online SFE-HPLC and GC-ECD or GC/MS. *Anal Chem* **66**(22), 4068–4073.
- Frazier, RG. (1994) Extraction of pesticide residues in soil and blueberry plants by supercritical fluid extraction. *Diss Abstr Int B* **54**(11), 5589.
- Heglund, DL, Tilotta, DC, Hawthorne, SB, Miller, DJ. (1994) Simple fiber-optic interface for on-line supercritical fluid extraction Fourier transform infrared spectrometry. *Anal Chem* **66**(20), 3543–3551.
- Gere, DR, Derrico, EM, Majors, RE. (1994) Sample preparation perspectives. SFE theory to practice – first principles and method development. II. *LC-GC Int* **7**(7), 370–372, 374–375.
- Thiebaud, HP, Knize, MG, Kuzmicky, PA, Felton, JS, Hsieh, DP. (1994) Mutagenicity and chemical analysis of fumes from cooking meat. *J Agric Food Chem* **42**(7), 1502–1510.
- Nam, K-S, King, JW. (1994) Supercritical fluid extraction and enzyme immunoassay for pesticide detection in meat products. *J Agric Food Chem* **42**(7), 1469–1474.
- Kollmannsberger, H, Nitz, S. (1994) The flavour-composition of supercritical gas extracts: III. Clove (*Syzygium aromaticum*). Ueber die Aromastoffzusammensetzung von Hochdruck-Extrakten: III. Gewuerznelken (*Syzygium aromaticum*). *Chem Mikrobiol Technol Lebensm* **16**(3/4), 112–123.
- Snyder, JM, King, JW. (1994) Oilseed volatile analysis by supercritical fluid and thermal desorption methods. *J Am Oil Chem Soc* **71**(3), 261–265.
- Hung, TV, Unger, MA. (1994) Applications of supercritical fluid extraction in the Japanese food industry. *Food Aust* **46**(5), 235–239.

- Saito, M., Yamauchi, Y., Okuyama, T. eds. (1994) *Fractionation by Packed-Column SFC and SFE. Principles and Applications*. VCH Publishers, Inc., New York, NY.
- Chester, T.L., Pinkston, J.D., Raynie, D.E. (1994) Supercritical fluid chromatography and extraction. *Anal Chem* **66**(12), 106R-130R.
- Snyder, J.M., King, J.W. (1994) Analysis of volatile compounds from supercritical extracted soybeans by headspace gas chromatography and thermal desorption of a polymer adsorbent. *J Sci Food Agric* **64**(3), 257-263.
- Santerre, C.R., Goodrum, J.W., Kee, J.M. (1994) Roasted peanuts and peanut butter quality are affected by supercritical fluid extraction. *J Food Sci* **59**(2), 382-386.
- Jimenez, J.J., Atienza, J., Bernal, J.L., Toribio, L. (1994) Determination of carbendazim in lettuce samples by SFE-HPLC. *Chromatographia* **38**(5/6), 395-399.
- Ibrahim-Abdullah, M., Young, J.C., Games, D.E. (1994) Supercritical fluid extraction of carboxylic and fatty acids from *Agaricus* spp. Mushrooms. *J Agric Food Chem* **42**(3), 718-722.
- Blanch, G.P., Ibanez, E., Herranz, M., Reglero, G. (1994) Use of a programmed temperature vaporizer for off-line SFE/GC analysis in food composition studies. *Anal Chem* **66**(6), 888-892.
- Vilegas, J.H.Y., Lancas, F.M., Vilegas, W. (1994) Application of a home-made supercritical fluid extraction system to the study of essential oils. *Flavour Fragrance J* **9**(1), 39-43.
- Reverchon, E., Ambruosi, A., Senatore, F. (1994) Isolation of peppermint oil using supercritical CO₂ extraction. *Flavour Fragrance J* **9**(1), 19-23.
- Seidel, V., Lindner, W. (1993) Universal sample enrichment technique for organochlorine pesticides in environmental and biological samples using a redesigned simultaneous steam distillation-solvent extraction apparatus. *Anal Chem* **65**(24), 3677-3683.
- Polesello, S., Lovati, F., Rizzolo, A., Rovida, C. (1993) Supercritical fluid extraction as a preparative tool for strawberry aroma analysis. *HRC* **16**(9), 555-559.
- Maxwell, R.J., Parks, O.W., Piotrowski, E.G. (1992) Improved SFE recovery of trace analytes from liver using an integral micrometering valve-SPE column holder assembly. *HRC* **15**(12), 807-811.
- Snyder, J.M., King, J.W. (1993) Supercritical fluid extraction of poultry tissues containing incurred pesticide residues. *J AOAC Int* **76**(4), 888-892.
- King, J.W. (1993) Analysis of fats and oils by SFE and SFC. *INFORM* **4**(9), 1089-1090, 1092-1095, 1097-1098.
- Berg, B.E., Hansen, E.M., Gjørven, S., Greibrokk, T. (1993) On-line enzymatic reaction, extraction, and chromatography of fatty acids and triglycerides with supercritical carbon dioxide. *HRC & CC*; **16**(6), 358-363.
- Vigh, L., Simandi, B., Deak, A., Kemeny, S., Toemoeskozei, S. (1993) Optimization of supercritical fluid extraction of corn germ oil in a multipurpose extractor. *Proceedings of the World Conference on Oilseed Technology and Utilization*.
- Johansen, H.R., Thorstensen, C., Greibrokk, T., Becher, G. (1993) On-line SFE-GC for determination of PCBs in human milk and blood serum. *HRC & CC*; **16**(3), 148-152.
- Taylor, S.L., King, J.W., Richard, J.L., Greer, J.I. (1993) Analytical-scale supercritical fluid extraction of aflatoxin B1 from field-inoculated corn. *J Agric Food Chem* **41**(6), 910-913.
- Taylor, S.L., King, J.W., List, G.R. (1993) Determination of oil content in oilseeds by analytical supercritical fluid extraction. *J Am Oil Chem Soc* **70**(4), 437-439.
- Tang, P.H., Ho, J.S., Eichelberger, J.W. (1993) Determination of organic pollutants in reagent water by liquid-solid extraction followed by supercritical fluid elution. *J AOAC Int* **76**(1), 72-82.
- Wigfield, Y.Y., Lanouette, M. (1993) Supercritical fluid extraction of the fortified residues of fluzifop-P-butyl (fusilade II) and its major metabolite, fluzifop-P, in onions. *J Agric Food Chem* **41**(1), 84-88.
- Reverchon, E., Senatore, F. (1992) Isolation of rosemary oil: comparison between hydrodistillation and supercritical CO₂ extraction. *Flavour Fragrance J* **7**(4), 227-230.
- Hopper, M.L., King, J.W. (1991) Enhanced supercritical fluid carbon dioxide extraction of pesticides from foods using pelletized diatomaceous earth. *J AOAC* **74**(4), 661-666.

- Artz, WE, Sauer, RM, Jr. (1992) An improved micro-extraction cell for supercritical fluid extraction and chromatography of fatty acids. *J Am Oil Chem Soc* **69**(4), 309–313.
- France, JE, King, JW. (1991) Supercritical fluid extraction/enzyme assay: a novel technique to screen for pesticide residues in meat products. *J AOAC* **74**(6), 1013–1016.
- Miles, WS, Quimby, BD. (1990) Characterization of sulfur compounds in spices using SFE-GC-AED. *Am Lab* **22**(11), 28F, 28H, 28J, 28L, '14.
- Nielsen, TJ, Jaegerstad, IM, Oeste, RE, Sivik, BTG. (1991) Supercritical fluid extraction coupled with gas chromatography for the analysis of aroma compounds absorbed by low-density polyethylene. *J Agric Food Chem* **39**(7), 1234–1237.
- Houben, RJ, Janssen, HGM, Leclercq, PA, Rijks, JA, Cramers, CA. (1990) Supercritical fluid extraction-capillary gas chromatography: on-line coupling with a programmed temperature vaporizer. *HRC & CC* **13**(10), 669–673.
- Cotton, NJ, Bartle, KD, Clifford, AA, Ashraf, S, Moulder, R, Dowle, CJ. (1991) Analysis of low molecular weight constituents of polypropylene and other polymeric materials using on-line SFE-SFC. *HRC & CC* **14**(3), 164–168.
- Majors, RE. (1991) Supercritical fluid extraction – an introduction. *LC-GC Int* **4**(3), 10, 12, 14, 16–17.
- Hedrick, JL, Taylor, LT. (1990) Supercritical fluid extraction strategies of aqueous based matrices. *HRC & CC* **13**(5), 312–316.
- Sandra, P, David, F, Stottmeister, E. (1990) Recovery studies by off-line SFE. *HRC & CC* **13**(4), 284–286.

EDITORIAL

Supercritical fluid techniques in analytical chemistry

This second issue of *Seminars in Food Analysis* is devoted to the application of supercritical fluid techniques in analytical chemistry. Supercritical fluids have woven their way into many traditional and new analytical and experimental techniques, such as chromatography, extraction, nuclear magnetic resonance spectroscopy, immunoassay, infrared spectroscopy, field flow fractionation, and thermo-optical absorption. They have been used in many hyphenated methods that read like an "alphabet soup": SFE-GPC, SFE-FTIR, SFE-GC-MS, SPE-SFE-GC, SFE-SFC-FTIR-MS, and SFC-ICP-MS. However, most analytical chemists or food analysts tend to identify the use of supercritical fluids for analysis with two major techniques: supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC). It should be noted that the first evidence of activity in the two above fields was initiated in 1962 and 1977 for SFC and SFE, respectively; however, it was not until the early 1980s that SFC became a commercial reality and in the very late 1980s for SFE. One application area in which both SFE and SFC have enjoyed considerable success is in the analysis of foods and agricultural products. For this reason we have dedicated the current issue of *Seminars in Food Analysis* to a state-of-the-art coverage on the use of supercritical fluids for the analysis of foods.

Contributions from six distinguished authors highlight this issue, covering distinct areas of application, a diverse array of target analytes, and other applicable analytical techniques besides SFE and SFC. The successful application of SFE for the assay of pesticides in fruits and vegetables has been elaborated on by Dr Steven Lehotay of USDA, using several different types of commercial instrumentation. The power of infrared (IR) spectroscopy combined with SFE for the characterization and quantification of lipid moieties is noted by Dr Philip Liescheski of Isco, a company that has been prominent in addressing the equipment needs of analysts using supercritical fluids in their research and methods development. Ms Christina Borch-Jensen of Denmark describes the use of SFE for oil and lipids analysis, noting that SFC is not a panacea for every separation problem that the food analyst faces.

Dr Alida Stolker's (Bilthoven, The Netherlands) contribution documents the role that analytical SFE has played in the creation of a low solvent methodology for drug and toxicant residue analysis. She and colleague Dr Robert Maxwell of USDA's Eastern Regional Research Center have been particularly active in the integration of sorbent technology into SFE-based protocols for the analyses of such analytes as anabolic steroids, sulfa drugs, and mycotoxins. Dr Beth Calvey of FDA's Center for Food Safety and Applied Nutrition details her experience with coupling SFC and SFE with FTIR and MS, showing their application to an extremely diverse and interesting array of food and toxicant analyses involving the packaging of migrants, phytochemical chemicals, and mycotoxins. Finally, Drs Fred Eller and Jerry King summarize the status of SFE methods for determining fat content in food stuffs, noting "... that things are not always as they seem ..." when defining and measuring fat content.

In summary, we hope the reader, and more importantly the analyst, will find the contents of this focus issue of relevance in their method development efforts. Supercritical fluid methodologies have much to offer the food analyst and can contribute to the protection of the environment and the laboratory worker. For this additional reason, they should be implemented into the analytical chemistry laboratory whenever possible.

Jerry W. King

*Food Quality & Safety Research Unit, National Center for Agricultural Utilization Research,
Agricultural Research Service, USDA, 1815 N. University Street, Peoria, IL 61604 USA*